Draining Lymph Node Cells of Contact-Sensitized Mice Induce Suppression of Contact Sensitivity

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The application of hapten to the skin of mice can induce contact sensitivity (CS). It has also been well established that draining lymph node (DLN) cells can induce CS to the hapten used for skin painting when injected into naïve mice. This is true for DLN cells recovered about 24 h after skin painting with hapten. It is unclear, however, whether DLN cells recovered shortly after hapten application have the same ability. By using an adoptive transfer assay system, we examined the ability of DLN cells recovered from mice at various times after skin painting with hapten to induce CS. DLN cells harvested 18–24 h after the application of fluorescein isothiocyanate (FITC) or 2,4-dinitrofluorobenzene (DNFB) induced strong CS when injected into naïve mice. DLN cells harvested 3–6 h after the application of FITC or DNFB induced either only weak or no CS but induced suppression of the subsequent immunization to the two haptens. The suppression was hapten-specific, MHC restricted, and associated with the appearance of splenic suppressor T lymphocytes. Analyses with antibodies and ultraviolet (UV) B radiation demonstrated that suppression-inducing cells in DLNs were Ia^+, Thy-1^-, and functionally UV-sensitive. These data suggest that epicutaneous sensitization with hapten first induces immunologically specific suppressor activity in the draining lymph nodes, whereas immunogenic activity becomes predominant later.

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Application of highly reactive haptens to the intact skin in experimental animals can easily induce contact sensitivity (CS). Circumstantial evidence indicates that not only the effector but also the suppressor circuits of CS can be activated in response to antigenic stimuli (Zembala *et al*, 1976, Sy *et al*, 1977, Toews *et al*, 1980). Although a number of studies have provided much information about the cellular and molecular events in activation of effector pathway (Stingl *et al*, 1978, Bigby *et al*, 1989, Galvin *et al*, 1992), relatively little is known about the mechanisms involved in activation of the suppressor circuit (Turk *et al*, 1972). Especially, the role of draining lymph nodes (DLNs) in suppressor mechamisms has not been investigated as much as that in the effector pathway. Macher and Chase (1969a, 1969b) demonstrated that if the skin site of guinea pigs that had been treated intradermally with hapten was removed shortly after the treatment, tolerance to that hapten was generated. If longer time had passed before the skin site was excised, immunization to the hapten developed. They concluded that tolerogenic antigens of CS were those that escaped early from the skin site and moved directly into bloodstream bypassing DLNs, because the principal pathway for escape was proved to be through the regional veins. The approach used by Macher and Chase was so intriguing that the role of DLNs in activation of suppressor circuits during contact sensitization was ignored for a long time, but their investigations did not completely exclude the possibility that the antigens that had arrived at DLNs before the skin site was excised also participated in the induction of tolerance. The purpose of the current study, therefore, was to determine whether DLNs play any part in the activation of suppressor immune response during the induction period of CS introduced by skin painting with hapten. The characteristics of the cell population in DLNs involved in the development of immunosuppression were also investigated.

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Abbreviations: CS, contact sensitivity; DLN, draining lymph node; LC, Langerhans cell; s.c., subcutaneous(ly); i.v., intravenous(ly).

MATERIALS AND METHODS

Animals Female BALB/c and C3H/HeN mice were obtained from Japan Clea (Shizuoka, Japan). Each experiment was performed with age-matched mice between 5 to 10 wk of age. The mice were maintained in filter-protected cages and given pelleted food and water *ad libitum*. Ambient lighting was controlled by automatic timers to provide 12-h light/dark cycles. Each experimental or control panel consisted of 5 to 10 mice.

Contact Sensitization Fluorescein isothiocyanate (FITC; Sigma, St. Louis, MO) solution, 0.5% (wt/vol), was prepared in a solvent composed of equal volumes of acetone and di-*n*-butyl phthalate (Wako Pure Chemical, Osaka, Japan). 2,4-Dinitrofluorobenzene (DNFB; Nacalai Tesque, Kyoto, Japan) was dissolved in acetone:olive oil (4:1) to prepare 0.5% and 0.2% solutions (wt/vol). For sensitization, 400 μl of 0.5% FITC or 25 μl of 0.5% DNFB solution was applied to shaved abdominal skin of the mice. Six days later, the animals were challenged by applying 10 μl of 0.5% FITC or 20 μl of 0.2% DNFB solution to ear surface. Ear thickness was measured with a dial thickness gauge (Ozaki, Tokyo, Japan) before and 24 h after challenge.

Assay for Induction of CS and Its Suppression Cell suspensions were prepared from the inguinal and mesenteric lymph nodes and spleen of mice sensitized 1, 3, 6, 12, 18, or 24 h earlier with sensitizing dose of FITC or DNFB solution on shaved abdominal skin. The cells were washed twice in RPMI 1640 medium and filtered through nylon mesh. Filtered cell suspensions (0.1 ml) containing 5×10^5, 1×10^6, 5×10^6, or 1×10^7 cells were injected subcutaneously (s.c.) into each hind footpad of syngeneic or

allogeneic naïve recipient mice. Six days later, the recipients were challenged on the ear with FITC or DNFB to test DLN cells for immunogenicity as described above. The mice were resensitized with FITC or DNFB through their shaved abdominal skin on the next day and challenged again 6 d later on the opposite ear.

Transfer of Suppression of CS with Spleen Cells Spleens were removed from the mice that were injected with DLN cells 7 d earlier and exhibited a decreased CS response. Single-cell suspensions were prepared from pooled spleens by teasing the spleens apart in Hanks' balanced salt solution. Clumps were removed by filtration through nylon mesh. The cells were washed once and refiltered, and 1×10^8 viable nucleated cells were injected intravenously (i.v.) into syngeneic recipients. Immediately thereafter the recipients were sensitized with FITC, and the CS response was measured 6 d later by ear challenge with FITC.

Treatment of Spleen and DLN Cells with Antibodies and Complement Spleen or DLN cells at a concentration of 2×10^7 cells per ml were incubated for 30 min at 4°C in monoclonal anti-Thy-1.2 antibody (Becton Dickinson, Mountain View, CA) diluted to 1:250 or in monoclonal anti-Ia^d antibody (Becton Dickinson) diluted to 1:200 in RPMI 1640 medium supplemented with 2% (vol/vol) fetal bovine serum. After washing three times in Hanks' balanced salt solution, the suspensions were incubated for 60 min at 37°C in a 1:8 dilution of rabbit complement. The cells were washed three times, counted, and injected s.c. or i.v. into recipient mice. Control cells were untreated or treated with complement alone.

Ultraviolet (UV) Irradiation and Freeze–Thaw Treatment of DLN Cells Saline cell suspensions (3 ml) containing 1×10^7 DLN cells per ml were placed in several 100-mm-diameter petri dishes and were exposed to 100 J per m^2 of UVB from a bank of seven unfiltered FL 20 SE-30 sunlamps (Toshiba Electric, Tokyo, Japan). About 75% of the energy emitted by these lamps falls within the UVB range (280–320 nm) with the peak emission at 305 nm. The dose of UV radiation was measured with a UV radiometer (UVR-305/365, Eizai, Tokyo, Japan). After irradiation, the cells were washed twice in RPMI 1640 medium and injected s.c. into recipient mice. As controls, some DLN cells were frozen at −70°C for 30 min and melted at 37°C for 30 min twice prior to injection.

Statistics Student t test was used to determine the statistical significance of differences in ear-thickness data between the experimental groups and control. A p value of less than 0.05 was considered significant.

RESULTS

DLN Cells Recovered 3–6 h after Painting with Hapten Induce Suppression of CS **Figure 1** shows the ear swelling response in mice receiving DLN cells collected at various time points after epicutaneous application of sensitizing dose of DNFB or FITC. The DLN are referred to as 1h-DLN, 3h-DLN, 6h-DLN, 12h-DLN, 18h-DLN, and 24h-DLN, depending on the time of collection after sensitization. The mice that received 3h-, 6h-, 12h-, or 24h-DLN cells from DFNB-painted donors exhibited a CS response to DNFB. No significant reaction was observed in the mice that received 1h-, 3h-, or 6h-DLN cells from FITC-sensitized donor mice. The mice injected with 18h-DLN cells, however, showed CS to FITC. To determine whether this hyporesponsiveness represents immunologic tolerance, all groups of recipient mice and control group were resensitized with epicutaneous application of DNFB or FITC on day 7, and the opposite ear was challenged 6 d later. As shown in **Fig 2**, mice injected with 3h- or 6h-DLN cells showed lower CS response compared to mice injected with no DLN cells in experiments using DNFB and FITC. These results suggest that DLN cells transferred from the donor mice painted with DNFB or FITC 3 and 6 h earlier were able to induce immunosuppression in the normal recipient mice.

Number of DLN Cells Required for Induction of Suppression A dose–response study was carried out with 6h-DLN cells from FITC-sensitized BALB/c mice to define suitable conditions for suppression induction **(Fig 3)**. Within the range of 5×10^5 to 5×10^6 cells, suppression was enhanced, with an increase in the number of DLN cells. The mice unexpectedly failed to induce suppression of CS when 1×10^7 DLN cells were introduced. Because of comparatively more significant suppressive effect, 5×10^6 6h-DLN cells from FITC-painted BALB/c strain mice were used for suppression induction in the subsequent experiments.

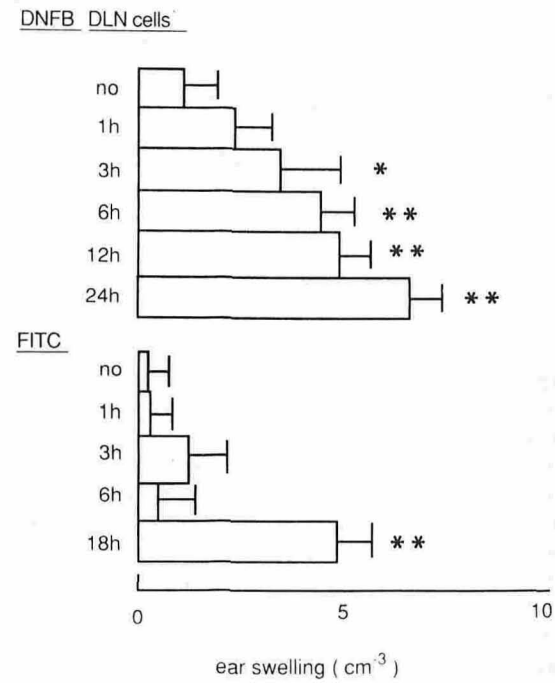


Figure 1. Transfer of DLN cells recovered from hapten-sensitized mice induce no, weak, or strong CS. DLN cells were obtained from BALB/c mice 1, 3, 6, 12, 18, or 24 h after skin painting with 25 μl of 0.5% DNFB or 400 μl of 0.5% FITC. Naïve recipient mice were injected s.c. with 5×10^6 DLN cells into each hind footpad and challenged on the ear with 20 μl of 0.2% DNFB or 10 μl of 0.5% FITC 7 d later. Data are mean ear swelling at 24 h after challenge. *Error bars*, SD (n = 5 to 10 mice per group). *, $p < 0.01$; **, $p < 0.001$ (compared to the control group—mice challenged only without DLN cell injection).

DLN-Cell–Induced Suppression Is Hapten-Specific The mice were injected s.c. with 5×10^6 6h-DLN cells recovered from FITC-painted donors and then resensitized with epicutaneous application of FITC. These mice showed a significantly lower CS response than the positive control group that received no DLN cells and only epicutaneous sensitization with FITC **(Fig 4)**. In contrast, the response of the experimental group to epicutaneous resensitization with DNFB was indistinguishable from that of a positive control group. These results indicate that the decreased CS response of mice injected with DLN cells from FITC-painted donors was specific for FITC.

Transfer of Suppression Induced by DLN Cells with Spleen Cells To determine whether suppression induced by DLN cells was associated with the presence of suppressor T cells in spleen, we injected normal recipient mice with one hundred million spleen cells harvested from the mice inoculated i.v. with DLN cells 7 d earlier. Immediately after injection, these mice were sensitized with epicutaneous application of FITC. As shown in **Fig 5**, mice given the spleen cells from DLN-cell–injected mice showed a suppressed CS response to FITC compared to the positive control. The suppressive effect was removed from the spleen cells by treatment with anti-Thy-1.2 antibody and complement before injection, but not by complement treatment alone. Thus, suppression induced by DLN cells appears to be associated with the presence of suppressor T cells in the spleen.

Mesenteric Lymph Nodes and Spleen Do Not Contain Cells with Suppressive Activity We next determined whether the other lymphoid tissues had also similar ability to induce downregulation. Mice were injected s.c. with 5×10^6 mesenteric lymph node cells or spleen cells prepared from the donor mice sensitized with

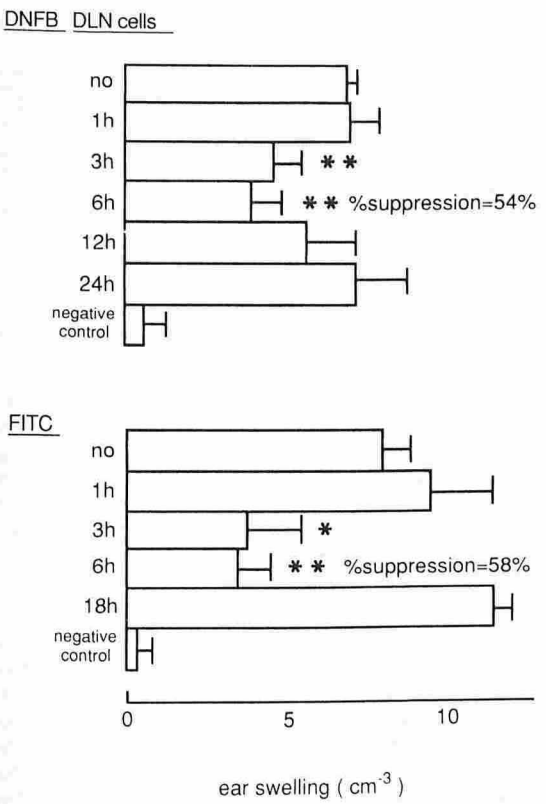


Figure 2. DLN cells recovered early after skin painting with hapten induce suppression of CS. After assessment of ear swelling depicted in **Fig 1**, resensitization was attempted by epicutaneous application of 25 μl of 0.5% DNFB or 400 μl of 0.5% FITC, and the opposite ear was challenged 6 d later. *, $p < 0.05$; **, $p < 0.01$ (compared to the control group—mice challenged only without DLN cell injection and resensitized with haptens). Percent suppression = [1 − (experiment − negative control/positive control − negative control)] × 100. *Error bars*, SD (n = 5 to 10 mice per group).

FITC 6 h earlier and then resensitized and challenged. The animals showed a CS response indistinguishable from that of the positive control group **(Fig 6)**, indicating that a cell population with

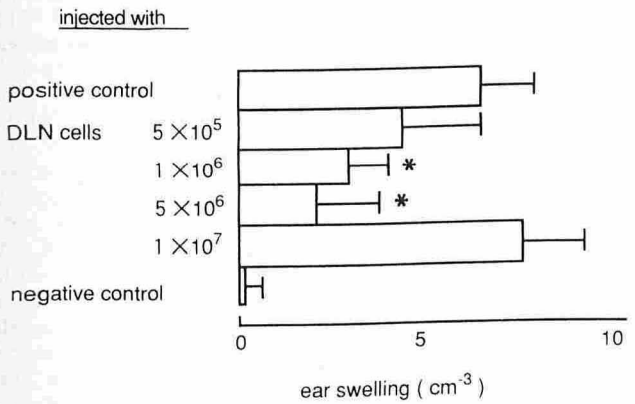


Figure 3. Suppression of CS by DLN cells is concentration-dependent. DLN cells were prepared from donor mice 6 h after painting with FITC. Recipient mice were injected s.c. with 5×10^5, 1×10^6, 5×10^6, or 1×10^7 cells and resensitized with FITC 7 d later. The ear was challenged 6 d after resensitization. *, $p < 0.05$ (compared to the positive control group—mice resensitized only without DLN cell injection). *Error bars*, SD (n = 5 to 10 mice per group).

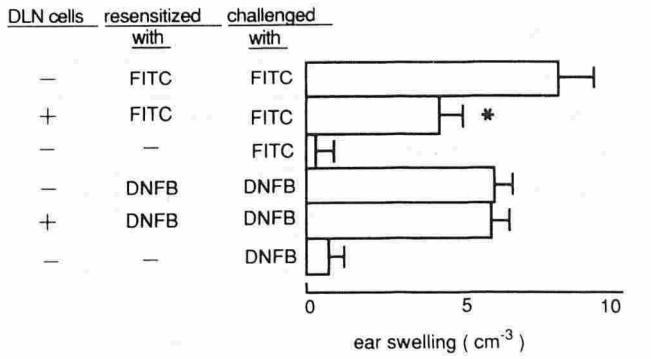


Figure 4. Suppression of CS induced by DLN cells is hapten-specific. Mice were injected with DLN cells obtained from FITC-painted donors, resensitized with FITC or DNFB, and challenged with the proper hapten. *, $p < 0.01$ (compared to the positive control group). *Error bars*, SD (n = 5 to 10 mice per group).

suppressive function does not exist in the mesenteric lymph nodes and spleen 6 h after contact sensitization with FITC.

Suppressive Activity of DLN Cells Is Major Histocompatibility Complex-Restricted BALB/c and C3H strain mice were injected, respectively, with syngeneic or alloeneic DLN cells recovered from FITC-sensitized donors. DLN cells from BALB/c mice suppressed CS response in BALB/c recipients but not in C3H recipients. Conversely, C3H DLN cells inhibited challenge reaction only in C3H recipients and not in BALB/c recipients **(Fig 7)**.

Suppressive Activity of DLN Cells Is Abrogated by Freeze-Thaw and Anti-Ia but Not by Anti-Thy-1.2 To determine whether viable cells are required for inducing suppression and to rule out the possibility that suppressive activity of DLN cells results from the transfer or reprocessing of antigen, DLN cells were killed by a freeze-thaw treatment. As shown in **Fig 8*a***, killing of DLN cells with this treatment abrogated their ability to mediate suppression. To obtain additional information on the phenotype of suppression-inducing cells in DLN cells, DLN cells recovered from FITC-sensitized mice were treated with anti-Ia^d or anti-Thy-1.2 antibody plus complement and then injected s.c. into naïve mice. Treatment of DLN cells with anti-Ia^d antibody and complement eliminated their ability to induce suppression of subsequent sensitization with FITC **(Fig 8*a*)**. Treatment with anti-Thy-1.2 antibody and complement had no effect **(Fig 8*b*)**. Thus, suppression-induc-

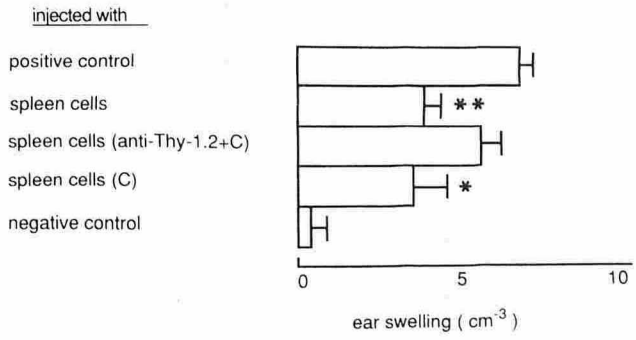


Figure 5. Suppression of CS induced by DLN cells is transferable with splenic suppressor T cells. Spleen cells were obtained from mice injected with DLN cells 7 d earlier. Naïve recipient mice were injected i.v. with 1×10^8 spleen cells and immediately thereafter resensitized with FITC. Spleen cells were treated with anti-Thy-1.2 antibody and complement (C) or C alone before transfer to recipients. Six days later the ear was challenged. *, $p < 0.01$; **, $p < 0.001$ (compared to the positive control). *Error bars*, SD (n = 5 to 10 mice per group).

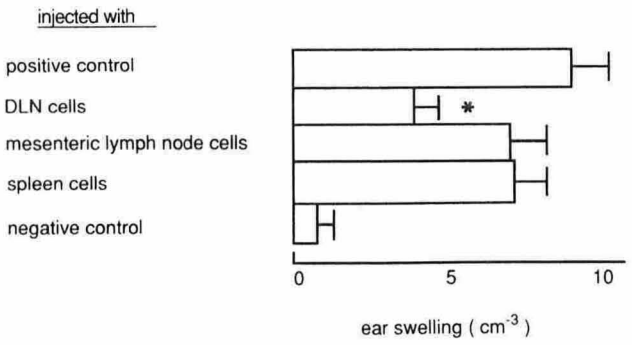


Figure 6. Mesenteric lymph node and spleen cells do not have suppressive activity. Five million cells prepared from DLNs, mesenteric lymph nodes, or spleen 6 h after painting with FITC were injected s.c. into recipient mice. The mice were resensitized and challenged with FITC. *, $p < 0.01$ (compared to the positive control group). *Error bars*, SD (n = 5 to 10 mice per group).

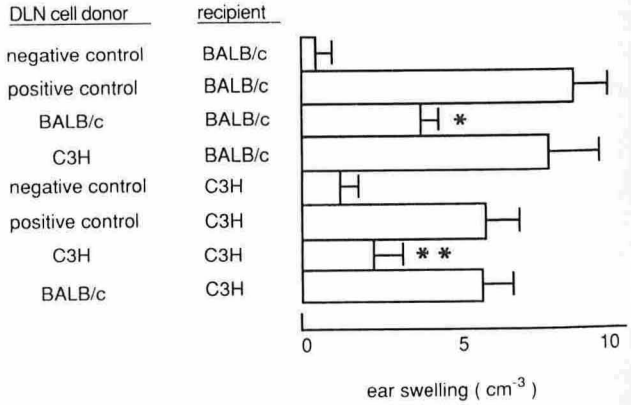


Figure 7. Suppressive activity of DLN cells is major histocompatibility complex-restricted. DLN cells obtained from BALB/c or C3H mice were injected into syngeneic and allogeneic mice, and the mice were resensitized and challenged. *, $p < 0.01$; **, $p < 0.001$ (compared to respective positive control groups). *Error bars*, SD (n = 5 to 10 mice per group).

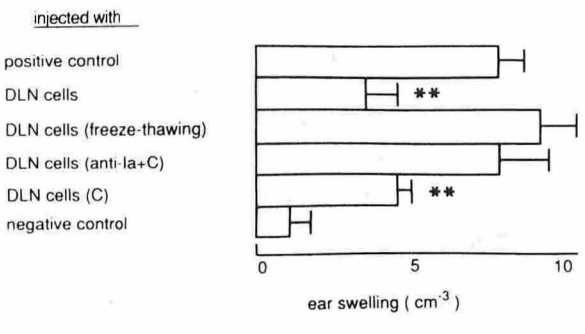
ing cells in DLN cells appear to be Ia-positive and Thy-1.2-negative.

Suppression-Inducing Cells in DLN Are Functionally UV-Sensitive There are several types of cells that are demonstrated to regulate a CS response (Toews *et al*, 1980, Granstein *et al*, 1987, Okamoto and Kripke, 1987). Some are resistant to UV radiation, and others are sensitive. To determine whether the suppression-inducing cells in DLNs are sensitive or resistant to UV radiation, DLN cells were irradiated with UV *in vitro* before transfer. In this experiment, only DNFB was used as hapten, because FITC is influenced by UV irradiation. Exposure of 6h-DLN cells to 100 J per m^2 of UVB radiation abrogated their ability to induce suppression of CS response **(Fig 9)**. There was no significant difference in cell viability or in the percentage of Ia-positive cells among viable cells between the irradiated 6h-DLN cells and the nonirradiated control as assessed with trypan blue exclusion and immunofluorescence using anti-Ia antibody (data not shown). It is therefore unlikely that the inability of UV irradiated 6h-DLN cells to induce suppression is due to cell death. The suppression-inducing cells in DLN appear to be functionally sensitive to UV.

DISCUSSION

It is well established that DLN cells recovered from mice about 20 h after sensitization with epicutaneous application of a hapten can transfer CS to the same hapten when injected into naïve mice (Thomas *et al*, 1980; Kripke and McClendon, 1986). In the current experiments, we demonstrated that DLN cells recovered from the donor mice 3–6 h after epicutaneous application of FITC and DNFB, when injected into naïve mice, induced suppression of subsequent sensitization with epicutaneous application of the two haptens. This suppression occurred via generation of hapten-specific suppressor T cells in the spleen. The formation of splenic suppressor T cells in mice given 6h-DLN cells is consistent with previously published data that demonstrated that suppressor cell activity appears in spleen when CS is induced in normal mice by the conventional skin painting technique (Chung *et al*, 1986). Our results suggest that DLN cells contain not only cells that activate the effector circuits but also cells that activate the suppressor circuits of CS. Furthermore, the suppressor circuits become predominant within several hours after epicutaneous application of the hapten, whereas the effector circuits become predominant later, about 20 h after hapten application.

The finding that although 1×10^6 to 5×10^6 6h-DLN cells induced suppression of CS, 1×10^7 6h-DLN cells failed to do so is contrary to our expectations. To clarify the mechanism of this phenomenon, mixtures of 5×10^6 6h-DLN cells and 5×10^5 to 5×10^6 24h-DLN cells were injected into naïve mice. In all cases, mixtures induced a CS response and did not show hyporesponsiveness to attempted sensitization with FITC (data not shown). This result indicates that the effect of sensitizing cells is dominant over



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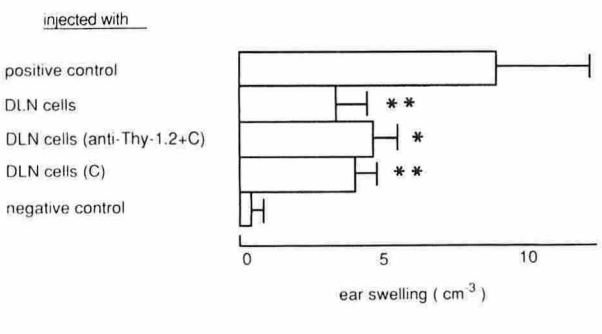

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Figure 8. Suppressive activity of DLN cells is abrogated by freeze-thaw and anti-Ia but not by anti-Thy-1 treatment. DLN cells were treated with freezing and thawing, anti-Ia antibody plus complement (C), or complement alone and injected into recipients *(a)*. DLN cells were treated with anti-Thy-1.2 antibody plus complement (C) or complement alone and injected into recipient mice *(b)*. The mice were resensitized and challenged. *, $p < 0.02$; **, $p < 0.01$ (compared to the positive control group). *Error bars*, SD (n = 5 to 10 mice per group).

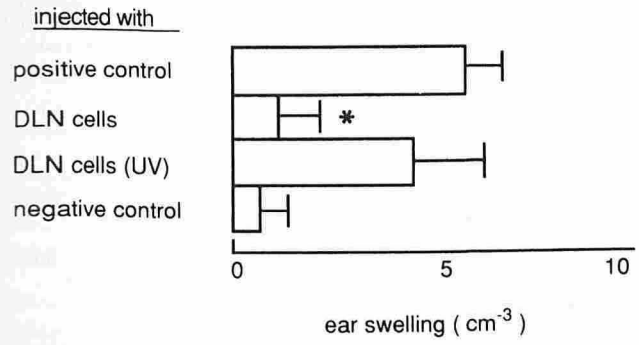

Figure 9. DLN cells involved in induction of suppression are UV-sensitive. DLN cells from DNFB-painted mice were exposed to UVB radiation (100 J per m^2) and injected into recipient mice. The mice were resensitized and challenged. *, $p < 0.001$ (compared to the positive control group). *Error bars*, SD (n = 5 to 10 mice per group).

the effect of tolerizing cells. We speculate that limiting amounts of sensitizing cells may be already present in 6h-DLN, so that when enough 6h-DLN cells are injected to override the effect of tolerizing cells, suppression of CS cannot be induced.

Only a few studies have been done to explore the tolerizing role of DLN. We have previously reported that subcutaneous administration of DLN cells obtained from mice 24 h after DNFB painting to UV-irradiated skin induced suppression of the subsequent immunization to DNFB (Oka *et al*, 1985). Okamoto and Kripke (1987) also showed that the induction of immunosuppression with DLN cells prepared from mice 18 h after FITC painting to the previously UV-irradiated skin correlates with the generation of suppressor T cells in spleen and that the inducer cells in DLNs are Thy-1^+ and Ia^- and may be derived from the Thy-1^+ dendritic epidermal cells. In the current study, depletion of Ia- but not Thy-1-bearing cells from DLN cells prevented these cells from inducing suppression of CS, indicating that the suppression-inducing cells were Thy-1^- and Ia^+. Therefore, the suppression-inducing cells that are found in DLNs within the first 3–6 h after hapten application to the intact skin are phenotypically distinct from those that are found in DLNs at later times after hapten application to the previously UV-irradiated skin.

Several types of cells are potential candidates for the suppression-inducing cells in DLNs. They include Langerhans cells (LCs), dermal Ia^+ cells, lymph node dendritic cells, and small B cells. LCs are antigen-presenting cells that play a crucial role in the induction and expression of CS (Silberberg-Sinakin *et al*, 1976; Toews *et al*, 1980). The suppression-inducing cells in DLNs that we studied were Ia^+ and Thy-1^- and, thus, were similar to LCs. Furthermore, these cells were functionally as UV sensitive as LCs. After epicutaneous application of sensitizing hapten, LCs pick up the hapten and rapidly migrate from the epidermis, via lymphatic vessels, to DLNs, where they interact with T cells (Cumberbatch and Kimber, 1990; Kripke *et al*, 1990). About 24 h after hapten painting, LCs acquire increased T-cell-stimulatory activity accompanied by the upregulation of membrane Ia antigen and adhesion/costimulatory molecules (Aiba and Katz, 1990; Aiba *et al*, 1993). In contrast, for the first 3 h after epicutaneous application of hapten, the expression of LC membrane Ia antigen is reduced because of an augmented internalization of the antigen (Becker *et al*, 1992a, 1992b). It is well demonstrated that when co-stimulation by accessory molecules on the antigen-presenting cells is lacking, T-cell proliferation does not occur and unresponsiveness to subsequent antigenic stimulus is induced (Jenkins *et al*, 1987; Tan *et al*,1993). Moreover, Simon *et al* (1991) indicated that UV radiation converts the function of LCs from one that induces an immunogenic signal to one that is tolerogenic, via alterations in co-stimulatory signals.

Thus, the data suggest that it is not unreasonable to speculate that LCs that migrate to DLNs early after hapten painting have the potential to deliver suppressive signals in the immunoregulation of CS, because they are not yet fully activated with respect to surface characteristics and functional properties. The suppression-inducing cells in DLNs in our study might be the LCs in such a stage. Granstein *et al* (1987) reported that high-density I-J major histocompatibility complex-restricted epidermal cells, when hapten-coupled and administered i.v. to mice, can activate transferable suppressor T cells and downregulate the subsequent induction of CS to that hapten. The authors suggested that these epidermal cells might be a subset of LCs or might represent a certain stage in the development of LCs.

It is also conceivable that the cells with suppression-inducing activity in DLNs of epicutaneously sensitized mice are not of epidermal origin. Our results do not exclude the possibility that Ia^+ cells in the dermis or lymph node dendritic cells may participate in the induction of immunosuppression of CS. Recently, perivascular dendritic Ia^+ cells, in the absence of LCs, have been shown to be capable of inducing CS when haptenated and injected intradermally (Tse and Cooper, 1990; Kurimoto *et al*, 1994). In addition, it has been indicated that hapten-derivatized Ia^+ dermal cells prepared from UV-exposed skin of UV-susceptible mice possess the ability to induce tolerance (Kurimoto *et al*, 1994). It is not yet clear, however, whether these cells in the UV-treated dermis are recruited or resident dermal cells. Macatonia *et al* (1987) have examined the cells involved in the development of CS in the DLNs of mice skin painted with FITC and concluded that low amounts of FITC binding selectively to veiled cells or lymph node dendritic cells in the first hours after exposure to antigen are not immunogenic but that LCs acquire high levels of antigen, enter the DLNs, and initiate immune responses. Although the ability of the lymph node dendritic cells with low levels of antigen to induce suppression of CS has not yet been examined, they are likely to have such ability because they are not fully activated. Small B cells should not be ignored, because antigen presentation by B cells to unprimed T cells can be tolerogenic. The mechanism of the induction of tolerance by small B cells is suggested to involve anergy or deletion of naïve antigen-specific T cells (Eynon and Parker, 1992), implying a difference from the mechanism of suppression mediated by antigen-specific suppressor T cells suggested by our results.

Our study shows that DLNs play a part in the activation of suppressor immune response during the induction period of CS induced by skin painting with two haptens, namely, FITC and DNFB, although the exact nature of the cells involved remains to be determined. Further isolation and identification of the related cell populations should be conducted to clarify the mechanisms of activation of the suppressor circuits in CS.

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REFERENCES

Aiba S, Katz SI: Phenotypic and functional characteristics of *in vivo*-activated Langerhans cells. *J Immunol* 145:2791–2796, 1990

Aiba S, Nakagawa S, Ozawa H, Miyake K, Yagita H, Tagami H: Up-regulation of α4 integrin on activated Langerhans cells: analysis of adhesion molecules on Langerhans cells relating to their migration from skin to draining lymph nodes. *J Invest Dermatol* 100:143–147, 1993

Becker D, Mohamadzadeh M, Reske K, Knop J: Increased level of intracellular MHC class II molecules in murine Langerhans cells following *in vivo* and *in vitro* administration of contact allergens. *J Invest Dermatol* 99:545–549, 1992a

Becker D, Neiβ U, Neis S, Reske K, Knop J: Contact allergens modulate the expression of MHC class II molecules on murine epidermal Langerhans cells by endocytotic mechanisms. *J Invest Dermatol* 98:700–705, 1992b

Bigby M, Vargas R, Sy MS: Production of hapten-specific T cell hybridomas and their use to study the effect of ultraviolet B irradiation on the development of contact hypersensitivity. *J Immunol* 143:3867–3872, 1989

Chung H-T, Burnham DK, Robertson B, Roberts LK, Daynes RA: Involvement of

prostaglandins in immune alterations caused by the exposure of mice to ultraviolet radiation. *J Immunol* 137:2478–2484, 1986

Cumberbatch M, Kimber I: Phenotypic characteristics of antigen-bearing cells in the draining lymph nodes of contact sensitized mice. *Immunology* 71:404–410, 1990

Eynon EE, Parker DC: Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. *J Exp Med* 175:131–138, 1992

Galvin F, Freeman GJ, Razi-Wolf Z, Hall W Jr, Benacerraf B, Nadler L, Reiser H: Murine B7 antigen provides a sufficient costimulatory signal for antigen-specific and MHC-restricted T cell activation. *J Immunol* 149:3802–3808, 1992

Granstein RD, Askari M, Whitaker D, Murphy G: Epidermal cells in activation of suppressor lymphocytes: Further characterization. *J Immunol* 138:4055–4062, 1987

Jenkins MK, Pardoll DM, Mizuguchi J, Chused TM, Schwartz RH: Molecular events in the induction of a non responsive state in IL-2 producing helper T lymphocyte clones. *J Immunol* 84:5409–5413, 1987

Kripke ML, McClendon E: Studies on the role of antigen-presenting cells in the systemic suppression of contact hypersensitivity by UVB radiation. *J Immunol* 137:443–447, 1986

Kripke ML, Munn CG, Jeevan A, Tang J-M, Bucana C: Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *J Immunol* 145:2833–2838, 1990

Kurimoto I, Arana M, Streilein JW: Role of dermal cells from normal and ultraviolet B-damaged skin in induction of contact hypersensitivity and tolerance. *J Immunol* 152:3317–3323, 1994

Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P: Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. *J Exp Med* 166:1654–1667, 1987

Macher E, Chase MW: Studies on the sensitization of animals with simple chemical compounds. XI.The fate of labeled picrylchloride and dinitrochlorobenzene after sensitizing injections. *J Exp Med* 129:81–102, 1969a

Macher E, Chase MW: Studies on the sensitization of animals with simple chemical compounds. XII.The influence of excision of allergenic depots on onset of delayed hypersensitivity and tolerance. *J Exp Med* 129:103–121, 1969b

Oka D, Nakagawa S, Kouchiyama A, Ueki H: The induction of tolerance in contact sensitivity by the injection of the regional lymph node cells from mice 1 day after painting the UV-B irradiated skin with DNFB to recipients. *Kawasaki Med J* 11:71–73, 1985

Okamoto H, Kripke ML: Effector and suppressor circuits of the immune response are activated *in vivo* by different mechanisms. *Proc Natl Acad Sci USA* 84:3841–3845, 1987

Silberberg-Sinakin I, Thorbecke GJ, Bear RL, Rosenthal SA, Berezowsky V: Antigen-bearing Langerhans cells in skin, dermal lymphatics and in lymph nodes. *Cell Immunol* 25:137–151, 1976

Simon JC, Tigelaar RE, Bergstresser PR, Edelbaum D, Cruz Jr PD: Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells, induction of specific clonal anergy in $CD4^+$ T helper 1 cells. *J Immunol* 146:485–491, 1991

Stingl G, Katz SI, Clement L, Green I, Shevach EM: Immunological functions of Ia-bearing epidermal Langerhans cells. *J Immunol* 121:2005–2013, 1978

Sy MS, Miller SD, Claman HN: Immune suppression with supraoptimal doses of antigen in contact sensitivity to DNFB. *J Immunol* 119:240–244, 1977

Tan P, Anasetti C, Hansen JA, Melrose J, Brunvand M, Bradshaw J, Ledbetter JA, Linsley PS: Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *J Exp Med* 177:165–173, 1993

Thomas WR, Edwards AJ, Watkins MC, Asherson GL: Distribution of immunogenic cells after painting with the contact sensitizers fluorescein isothiocyanate and oxazolone. Different sensitizers from immunogenic complexes with different cell populations. *Immunology* 39:21–27, 1980

Toews GB, Bergstresser PR, Streilein JW, Sullivan S: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresposiveness follows skin painting with DNFB. *J Immunol* 124:445–453, 1980

Tse Y, Cooper KD: Cutaneous dermal Ia+ cells are capable of initiating delayed type hypersensitivity responses. *J Invest Dermatol* 94:267–272, 1990

Turk JL, Parker D, Poulter LW: Functional aspects of selective depletion of lymphoid tissue by cyclophosphamide. *Immunology* 23:493–501, 1972

Zembala M, Asherson GL, Nowolski J, Mayhew B: Contact sensitivity to picryl chloride: the occurrence of B suppressor cells in the lymph node and spleen of immunized mice. *Cell Immunol* 25:266–278, 1976